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Stereoselective production of (*S*)-1-aralkyl- and 1-arylethanols by freshly harvested and lyophilized yeast cells

Balázs Erdélyi,[a,*] Antal Szabó,[a] Gábor Seres,[b] László Birincsik,[a] József Ivanics,[c] Gábor Szatzker[d] and László Poppe[d]

[a]*Fermentation Pilot Plant, IVAX Drug Research Institute Ltd, PO Box 82, H-1325 Budapest, Hungary*
[b]*Analytical Department, IVAX Drug Research Institute Ltd, PO Box 82, H-1325 Budapest, Hungary*
[c]*Synthetic Pilot Plant, IVAX Drug Research Institute Ltd, PO Box 82, H-1325 Budapest, Hungary*
[d]*Institute for Organic Chemistry and Research Group for Alkaloid Chemistry, Budapest University of Technology and Economics, Gellért tér 4., H-1111 Budapest, Hungary*

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Abstract—Substituted (*S*)-1-phenyl- **2a**–**h** and (*S*)-1-benzyl-propan-2-ols **4a** and **b**, and (*S*)-1-phenylethanol **6** were produced from prochiral ketones **1a**–**h**, **3a**,**b** and **5** by reductions with freshly harvested *Zygosaccharomyces rouxii* and *Debaryomyces hansenii* cells. The bioreductions were also performed by lyophilized cells. Comparison of the secondary alcohols from the bioreductions **2b**–**e**,**g**,**h** and **4a** and authentic (*S*)-alcohols (*S*)-**2b**–**e**,**g**,**h** and (*S*)-**4a** synthesized from enantiopure (*S*)-methyloxirane **7** proved the absolute configuration of the products.

1. Introduction

Prochiral aromatic ketones and the optically active forms of the corresponding secondary alcohols are essential building blocks for different pharmaceuticals and fine chemicals.[1] Microbial reduction of prochiral ketones possesses several advantages: regio- and enantioselectivity, in vivo cofactor regeneration, mild reaction conditions and environmentally friendly operation. There are few disadvantages to using whole cells as a biocatalyst: large fermenter capacity, difficulties in the course of process development and scale-up.[2] Whole cells frequently possess more dehydrogenases, which can be responsible for the production of the non-required enantiomer or even other by-products. In addition, the long-term storage of the biomass often suffers from a decrease in the carbonyl reductase activity. Even when storing the biomass in a refrigerated room, significant loss in enzyme activity is still observed and in some instances, the products were degraded or some by-product(s) were formed.[3]

Yeasts *Zygosaccharomyces rouxii*, *Mortierella isabellina*, *Candida famata* and *Candida guilliermondii* were found as efficient biocatalysts for the enantioselective reduction of 3,4-methylenedioxyphenylacetone **1a**.[4] The stereoselective bioreduction of **1a** is the key step in the synthesis of Talampanel, an antiepileptic drug candidate (Fig. 1).[5] *Z. rouxii* has been chosen as a biocatalyst for process development and scale-up, because it tolerated higher substrate and product concentrations than the other investigated species and it could regenerate the cofactor anaerobically.[6] The specific reductase activity of *Z. rouxii* was found to be very sensitive to the cell cultivation and storage conditions.[7] Molinari et al. described the stereoselective reductions of different methyl ketones using lyophilized yeasts of seven different species.[8] In several cases, the lyophilized cells were found to be more effective biocatalysts than fresh cells without significant alteration in stereoselectivity.

C. famata was described as an efficient biocatalyst in bioreductions.[4] We supposed that the teleomorph of this species, *Debaryomyces hansenii,* may also be useful for the enantioselective reduction of prochiral aromatic ketones.

Herein we have reported the bioreductions of different prochiral aryl- and aralkyl ketones to their corresponding

* Corresponding author. Tel.: +36 1 399 3330; fax: +36 1 399 3321; e-mail: balazs.erdelyi@idri.hu

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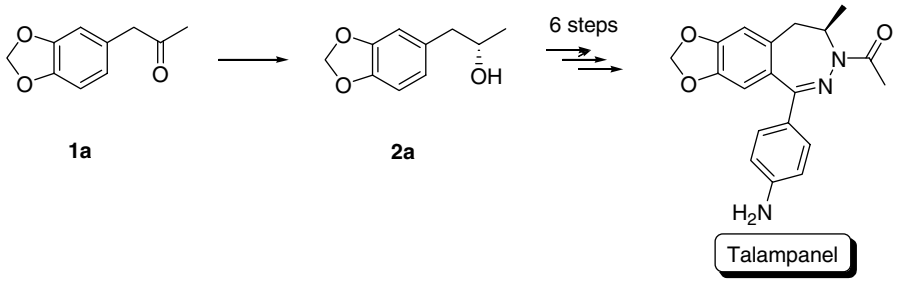
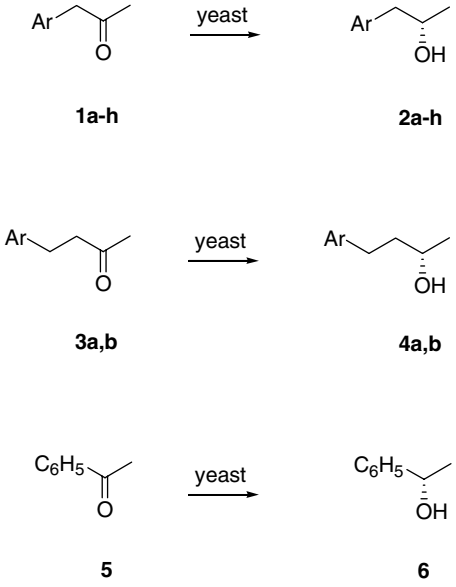


Figure 1. Abbreviated scheme for the synthesis of Talampanel.



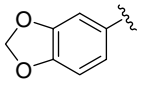
Comp.	Ar
1,2a	
b	C_6H_5
c	2-MeO-C_6H_4
d	3-MeO-C_6H_4
e	4-MeO-C_6H_4
f	3,4-$(MeO)_2$-C_6H_3
g	2,4-$(MeO)_2$-C_6H_3
h	4-Cl-C_6H_4
3,4a	C_6H_5
b	4-MeO-C_6H_4

Figure 2. Bioreduction of aralkyl-methyl **1a**–**h** and **3a**,**b** and aryl-methyl ketones **5** by yeasts.

alcohols (Fig. 2), which are possible intermediates in the syntheses of further drug candidates. First, several yeasts, which had been used in our institute (IVAX) in the bioreduction of other compounds, were tested. The enzyme activity and enantioselectivity of the most promising *Z. rouxii* and *D. hansenii* cells have been further investigated. An increase in the total concentration of the bioreduction was not the subject of our investigation, since it could be easily increased up to 40 g/l by using proper solid adsorbent (e.g., XAD-7 resin in bioreduction of **1a**).[6]

Finally, lyophilization of the yeast cells and the usefulness of the lyophilized biocatalysts in bioreductions have also been studied. Thus, the carbonyl reductase activities present in the lyophilized and fresh cells were compared.

2. Results and discussion

Four prochiral ketones **1a**, **1b**, **1e** and **3a** were chosen to compare the reductase systems of the yeast strains involved in our experiments. *Z. rouxii* and *D. hansenii* were found as promising catalysts (Table 1). Remarkable differences were found in the reductase activity among the three *Z. rouxii* strains. The strain purchased from ATCC reduced the ketones most efficiently, with the exception of **3a**. *D. hansenii* also proved to be an effective biocatalyst, whereas the other strains resulted in low yields. Therefore, *Z. rouxii* (ATCC 14462) and *D. hansenii* were selected for the further experiments.

Table 1. Reduction of aralkyl-methyl ketones **1a**,**b**,**e** and **3a** by several yeasts

Yeast	Yield (%)			
	2a	**2b**	**2e**	**4a**
Zygosaccharomyces rouxii (ATCC 14462)	90	>99	82	28
Z. rouxii (NCAIM Y00180)	48	66	65	26
Z. rouxii (IDR-503)	79	65	78	82
Debaryomyces hansenii	>99	78	67	57
D. carsonii	69	88	71	85
Candida guilliermondii	28	41	33	53
Pichia kijperi	60	59	66	60
Saccharomyces bayanus	21	22	16	15

Next, cosubstrates for cofactor regeneration required in bioreductions were tested with the best strains. In accordance with earlier reports, glucose was found to be the best cosubstrate for the bioreductions with *Z. rouxii*. In addition, sucrose has been found as an alternative cosubstrate of *Z. rouxii*. Experiments were carried out

to determine the most advantageous cosubstrate of *D. hansenii*. Propan-2-ol was found to be better than glucose and sucrose (Table 2). It has been observed that the different carbohydrates and alcohols as cosubstrates had no impact on the enantiomeric composition of the product. As propan-2-ol proved to be the most effective cosubstrate for *D. hansenii*, it was used in the further experiments.

Table 2. The effect of cosubstrate on the reductase activity of *D. hansenii*

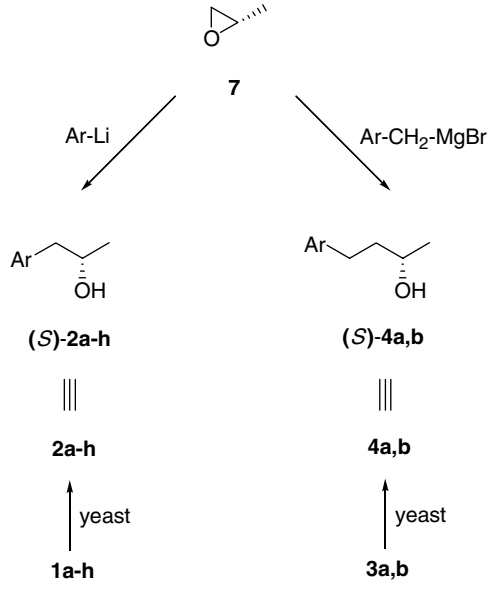
Cosubstrate	Yield (%)	
	(*S*)-**2a**	(*S*)-**4a**
Glucose	78	76
Sucrose	76	78
Glycerol	54	32
Sorbite	33	33
Methanol	32	23
Ethanol	30	15
Propan-1-ol	20	11
Propan-2-ol	>99	86
Butan-1-ol	5	7
Butan-2-ol	5	9

Our next experiments indicated that all ketones **1a**–**h**, **3a**,**b** and **5** could be reduced by *Z. rouxii* and *D. hansenii* (Fig. 2). Table 3 shows that *D. hansenii* possessed wider substrate tolerance than *Z. rouxii*. This can be seen by the reduction of *ortho*-substituted phenylacetones **1a** and **1g**, which were reduced with lower yield by *Z. rouxii*. Reductions with *D. hansenii* as biocatalyst resulted in yields between 57% and 100%; lower yields were achieved in the reductions of the bulky disubstituted ketones **1f** and **1g**, and when the carbonyl moiety is more remote from the aryl group **3a** and **b**. The degree of stereoselectivity in bioreductions with both strains was usually high, particularly when the aromatic ring of the 1-arylpropan-2-ones was substituted at the *para*-position (**1a**, **1f** and **1h**). In the cases of ketones with non-substituted phenyl ring **1b** and **3a**, a significant decrease in enantioselectivity was observed. The fact that the non-substituted acetophenone **5** was reduced to enantiomerically pure (*S*)-1-phenylethanol **6** indicates that the close vicinity of an even non-substituted aromatic ring to the reduction centre can also provide sufficient steric difference and thus can result in high stereoselectivity. Unsurprisingly, the degree of stereoselectivity decreased in the bioreductions of ketones bearing the aryl moiety at more distant position **3a** and **3b**.

Table 3. The carbonyl reductase activity of freshly harvested *Z. rouxii* and *D. hansenii* cells in reduction of aralkyl-methyl **1a**–**h** and **3a**,**b** and aryl-methyl ketones **5**

Product	*Z. rouxii* (ATCC 14462)		*D. hansenii*	
	Yield (%)	ee (%)	Yield (%)	ee (%)
(*S*)-**2a**	90	>99	>99	>99
(*S*)-**2b**	>99	82	78	77
(*S*)-**2c**	15	7	63	88
(*S*)-**2d**	85	87	71	93
(*S*)-**2e**	82	>99	67	95
(*S*)-**2f**	31	>99	58	>99
(*S*)-**2g**	17	80	58	>99
(*S*)-**2h**	84	>99	78	>99
(*S*)-**4a**	28	66	57	59
(*S*)-**4b**	33	82	58	80
(*S*)-**6**	37	>99	63	>99

The absolute configurations of the known secondary alcohols from the bioreductions with *Z. rouxii* and *D. hansenii* **2a**, **2f** and **6** were found to be *S*, in accordance with the Prelog-rule. Although on this basis the absolute configurations of all the secondary alcohols produced by bioreductions with these two strains can be supposed as *S*, we unambiguously determined the absolute configuration of the less or non-characterized secondary alco-



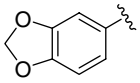
Comp.	Ar
1,2a	
b	C_6H_5
c	2-MeO-C_6H_4
d	3-MeO-C_6H_4
e	4-MeO-C_6H_4
f	3,4-$(MeO)_2$-C_6H_3
g	2,4-$(MeO)_2$-C_6H_3
h	4-Cl-C_6H_4
3,4a	C_6H_5
b	4-MeO-C_6H_4

Figure 3. Comparison of the secondary alcohols **2a**–**h** and **4a**,**b** obtained by bioreductions to (*S*)-aralkyl-methyl carbinols (*S*)-**2a**–**h** and (*S*)-**4a**,**b** synthesized from (*S*)-propylene oxide **7**.

hols **2b–e,g,h** and **4a**. For this, authentic (*S*)-alcohols (*S*)-**2b–e,g,h** and (*S*)-**4a** were synthesized from enantiopure (*S*)-propylene oxide **7** (Fig. 3) and compared with the corresponding products of the bioreductions **2b–e,g,h** and **4a** by HPLC on a chiral stationary phase.

As the separation of the cell harvesting and bioreduction step in time and/or location might be important for industrial application, lyophilization and applicability of the lyophilized cells as biocatalysts were studied next. Dry-ice has been found as a suitable freezing medium for lyophilization of *Z. rouxii* cells (Table 4). The number of the survived cells and the productivity (i.e., yield of **1a**) did not decrease significantly while the degree of stereoselectivity remained unaltered.

Table 4. The effect of different freezing methods on the reductase activity of *Z. rouxii* in production of (*S*)-**2a**

Freezing method	Survived cells (%)	Yield (%)	ee (%)
Dry ice in acetone (ca. −79 °C)	85–90	90	>99
Liquid nitrogen (−200 °C)	60–70	85	>99
Slow freezing (−80 °C)	40–50	67	>99

The investigation of lyophilized and rehydrated (after a month storage at 4 °C) cells of the two selected yeast strains (*Z. rouxii* and *D. hansenii*) have been extended to the reduction of ketones **1a–h**, **3a,b** and **5**. In most cases, the yields and enantiomeric purities did not change significantly (Tables 3 and 5). In the case of *Z. rouxii* however, an increase in the stereoselectivity was observed for the reduction of ketones **1g** and **3b** (Tables 3 and 5; the ee values increased from 80% and 82% to 99% and 93%, respectively). In the case of bioreductions with rehydrated *D. hansenii* cells (Tables 3 and 5) the most significant change has been found in reduction of benzylacetones **3a** and **3b** (the ee values increased from 59% and 80% to 79% and 95%, respectively). This can be rationalized by assuming selective destruction of concurrent carbonyl reductas(es) having a higher affinity towards the benzylacetones during lyophilization of the *D. hansenii* cells either with anti-Prelog stereoselectivity in reduction or with Prelog selectivity but catalyzing back-oxidation.

Table 5. The carbonyl reductase activity of the lyophilized and rehydrated *Z. rouxii* and *D. hansenii* cells in reduction of aralkyl-methyl **1a–h** and **3a,b** and aryl-methyl ketones **5**

Product	*Z. rouxii* (ATCC 14462)		*D. hansenii*	
	Yield (%)	ee (%)	Yield (%)	ee (%)
(*S*)-**2a**	87	>99	77	>99
(*S*)-**2b**	90	85	93	79
(*S*)-**2c**	9	5	75	93
(*S*)-**2d**	69	87	75	93
(*S*)-**2e**	80	>99	77	>99
(*S*)-**2f**	31	>99	64	>99
(*S*)-**2g**	14	>99	67	>99
(*S*)-**2h**	87	>99	88	>99
(*S*)-**4a**	27	57	84	79
(*S*)-**4b**	29	93	63	95
(*S*)-**6**	30	>99	58	>99

The successful lyophilization experiments with the two yeast strains resulting in beneficial biocatalysts initialized further attempts for drying the yeast biomass on an industrial scale in different air-drier equipments.

3. Conclusion

The yeast strain *Z. rouxii* ATCC 14462 applied in the bioreduction of **1a** (the key step in Talampanel synthesis) possesses carbonyl reductase activity to produce (*S*)-alcohols **2b**, **2e** and **2h** in good yields and with excellent enantioselectivities. Bioreduction of a few aryl- and aralkyl-methyl ketones can also be performed by *D. hansenii* cells with consequent stereopreferences [(*S*)-alcohols] and appropriate yields. The *ortho*-substituted phenylacetones **1c** and **1g**, benzylacetones **3a** and **3b** and acetophenone **5** can be reduced in sufficient yields and enantiomeric purities (except of **4b**) with *D. hansenii* cells as well.

Lyophilization, by producing an easy-to-store-and-transport whole cell biocatalyst, enables (even on pilot plant scale) the separation of the biomass production and biotransformation steps in time and place.

4. Experimental

4.1. Materials and methods

4.1.1. Reagents and solvents. Bromobenzene, 4-bromoanisole, 1-bromo-4-chlorobenzene, 4-(4-methoxyphenyl)-2-butanone **3b** and acetophenone **5** were products of Fluka AG. Anisole, 3-bromoanisole, 1-bromo-2,4-dimethoxybenzene, 2-, 3- and 4-methoxyphenylacetones **1c**, **d** and **e**, respectively, (2,4-dimethoxyphenyl)- and (3,4-dimethoxyphenyl)acetones **1f** and **g**, respectively, racemic- and (*S*)-propylene oxide *rac*- and (*S*)-**7** and butyllithium (2.5 M in hexane) were purchased from Aldrich. 4-chlorophenylacetone **1h** was synthesized in our institute (IVAX). All synthetic reactions involving organometallic species were performed under argon atmosphere. All solvents were freshly distilled prior their use.

4.1.2. Biocatalysts. Whole cells of *Z. rouxii* from three different collection [American Type Culture Collection (ATCC) 14462, National Collection of Agricultural and Industrial Microorganisms (NCAIM) Y00180 and Institute for Drug Research (IDR) 503], *D. hansenii* (NCAIM Y00468), *C. guilliermondii* (IDR 425), *Saccharomyces bayanus* (NCAIM Y01081), *D. carsonii* (IDR 513) and *Pichia kijperi* (IDR 514) were used as biocatalysts.

4.1.2.1. Production of the biocatalysts. *Z. rouxii* was maintained and cultured as it was described elsewhere.[7] The other yeasts were maintained on YM agar contained yeast extract (0.3%), malt extract (0.3%), peptone (0.5%) glucose (1.0%) and agar (2.0%). The yeast cells were cultured in sterilized YMT medium in a 10-l working volume fermentor. YMT cultivation medium contained yeast extract (1.2%), malt extract (1.2%), peptone

(2.0%), glucose (4.0%) and P2000 antifoam agent (0.1%). The bioreactor was operated at 26 °C with an agitation set at 450 rpm, an aeration of 0.5 vvm air flow via a submerged sparger and a back pressure of 0.2 bar. The pH and the dissolved oxygen tension were measured. The cells were separated from the 24-h old broth with a laboratory centrifuge at 3000 rpm for 20 min and the supernatant removed. The cell paste contained about 25% total dry matter was used as biocatalyst.

4.1.2.2. Lyophilization and rehydration of the yeast cell pastes. The cell paste was used directly as biocatalyst or was lyophilized without washing. Cell pastes were lyophilized with Virtis Sentry 5L type dryer for 24 h, then the cell powders stored at 4 °C for a month.

The lyophilized cells were rehydrated in pH 7.0 buffer solution containing 3% glucose at 28 °C for 1 h and centrifuged again with the same parameters as described in Section 4.1.2.1. The viable cell number was checked with published microbial methods.[7]

4.1.3. Analytical methods. The NMR spectra were recorded in $CDCl_3$ on a Bruker BRX-300 spectrometer (at 300 MHz for ^1H and 75 MHz for ^{13}C spectra) and are reported in parts per million on the δ scale. IR spectra (film) were taken on a Specord 2000 Series spectrophotometer and the wave numbers of the absorptions are reported in cm^{-1}. GC analyses were carried out on HP 5890 or Agilent 4890D instruments equipped with FID detector and Lipodex E column (25 m × 0.25 mm; octakis-(2,6-di-*O*-pentyl-3-*O*-butyryl)-γ-cyclodextrin phase; Macherey-Nagel) or BetaDex 225 column (30 m × 0.25 mm; 2,3-di-*O*-acetyl-6-*O*-TBDMS-β-cyclodextrin phase; Supelco) using H_2 carrier gas (injector: 250 °C, detector: 250 °C, head pressure: 12 psi, 50:1 split ratio). Enantiomer separations by HPLC were performed on a ChiraGrom2 microbore column (250 × 2 mm × 4 μm; cellulose carbamate type chiral phase; Grom Analytik). The HPLC system controlled by Millennium2 software contained a Waters 510 pump and a Waters 486 detector. The mobile phase was a hexane/2-propanol mixture consisting of 2–10% 2-propanol at a flow rate of 0.5 ml/min. Optical rotations were determined on a Perkin Elmer 241 polarimeter. TLC for the synthetic reactions was carried out on Kieselgel 60 F_{254} (Merck) sheets. Spots were visualized by treatment with 5% ethanolic phosphomolybdic acid solution and heating of the dried plates.

The conversion rate in the bioreductions was measured by TLC (CAMAG TLC Linomat and Scanner).[9] Samples of 2, 4 and 8 μl and 2, 4 and 8 μl of reference alcoholic solution (1000 μg/ml) were applied onto the plate in 3 mm bands with a distance of 9 mm between the bands. Plates were developed in a Camag twin trough chamber using toluene–methylene chloride–tetrahydrofuran (92:4:4 v/v). Migration distance was 70 mm. Plates were scanned in reflectance mode at 220–290 nm. Concentrations of the substrate ketones (S) and of the product alcohols (P) were obtained from a three-plot calibration curve of references. Conversion rates (%) were determined as follows: P/S × 100.

4.2. Bioreduction of the prochiral ketones 1a–h, 3a,b and 5

Both fresh and lyophilized/rehydrated cells were used in the bioreduction as follows: centrifuged cell paste (8.0%), ketone **1a**–**h**, **3a**,**b** or **5** (10 mM), glucose (1.0 w/v%) or 2-propanol (1%, only to *D. hansenii* cells) were completed with water to 25 ml in 100 ml Erlenmeyer flasks. The mixtures were shaken at 28 °C for 1 h (325 rpm) than the whole mixture was extracted with 25 ml of ethyl acetate.

4.3. Synthesis of the (*S*)-aralkyl-methyl-carbinols (*S*)-2a–h by ring opening of (*S*)-propylene oxide 7 with aryl lithium compounds

To the stirred solution of the corresponding aryl bromide (2 mmol, for production of **2b**,**d**,**e**,**g**,**h**) or anisole (2 mmol, for production of **2c**) in THF (5 ml) butyllithium (2.1 mmol, 2.5 M in hexane) was added below −65 °C and resulting mixture stirred at about −70 °C for 20 min. Then (*S*)-propylene oxide (*S*)-**7** (3 mmol, 0.17 g, 0.21 ml) was added under −50 °C and mixture stirred at this temperature for 20 min. Then, the temperature was raised up to rt (within about 30 min), and the reaction mixture stirred for 1 h. The reaction mixture was quenched by the addition of brine (2 ml) and diethyl ether (10 ml). After separation of the forming layers, the organic phase was washed with water (3 ml), 5% HCl solution (3 ml), saturated $NaHCO_3$ solution (3 ml) and brine (3 ml), successively. The organic solution was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel; hexane–acetone 10:1) to yield the corresponding secondary alcohol (*S*)-**2b**–**e**,**g**,**h**.

The corresponding racemic alcohols *rac*-**2b**–**e**,**g**,**h** were synthesized from aryl bromides or anisole (5 mmol) using butyllithium (5.3 mmol) and racemic propylene oxide (*rac*-**7**, 7.5 mmol) in similar manner.

4.3.1. Racemic 1-phenylpropan-2-ol *rac*-2b. Yield: 72%; IR: 3360, 2968, 1496, 1476, 1080, 940, 744, 700; ^1H NMR: 1.27 (3H, d, $J = 6.1$ Hz, CH_3), 2.41 (1H, br s, OH), 2.70–2.85 (2H, m, CH_2), 4.01–4.08 (1H, m, O–CH), 7.23–7.37 (5H, m, Ar–H), ^{13}C NMR: 22.86, 45.89, 69.07, 126.60, 128.67, 129.551, 138.60. Anal. Calcd for $C_9H_{12}O$: C, 79.37, H, 8.88. Found: C, 79.15, H, 8.76.

4.3.2. (*S*)-1-Phenylpropan-2-ol (*S*)-2b. Yield: 71%; ee: >99% (GC); $[\alpha]_D^{25} = +42.2$ (*c* 1.0, $CHCl_3$) {lit.:[10] $[\alpha]_D = +41.8$ (*c* 2.15, $CHCl_3$)}; ^1H NMR spectrum agreed with the reported data.[11] The IR, ^1H and ^{13}C NMR spectra of (*S*)-**2b** were indistinguishable from that of *rac*-**2b**.

4.3.3. Racemic 1-(2-methoxyphenyl)propan-2-ol *rac*-2c. Yield: 79%; IR: 3389, 2968, 1573, 1469, 1272, 1180, 1047, 944, 791; ^1H NMR: 1.1 (3H, d, $J = 6.2$ Hz, CH_3), 1.90 (1H, br s, OH), 3.15–3.20 (1H, m, CH_2), 3.50–3.68 (1H, m, CH_2), 3.77 (1H, s, O–CH_3), 3.92–4.00 (1H, m, O–CH), 6.81–6.91 (2H, m, $Ar_{3,5}$–H),

7.13–7.20 (2H, m, $Ar_{4,6}$–H); ^{13}C NMR: 23.37, 40.88, 55.68, 70.70, 111.13, 121.22, 124.80, 128.00, 129.50, 157.79. Anal. Calcd for $C_{10}H_{14}O_2$: C, 72.26, H, 8.49. Found: C, 72.03, H, 8.55.

4.3.4. (*S*)-1-(2-Methoxyphenyl)propan-2-ol (*S*)-2c. Yield: 77%; ee: >99% (GC); $[\alpha]_D^{25} = +39.8$ (*c* 1.0, $CHCl_3$); ^1H NMR spectrum agreed with the reported data.[12] The IR, ^1H and ^{13}C NMR spectra of (*S*)-**2c** were indistinguishable from that of *rac*-**2c**.

4.3.5. Racemic 1-(3-methoxyphenyl)propan-2-ol *rac*-2d. Yield: 76%; IR: 3384, 2968, 1584, 1488, 1260, 1152, 1044, 944, 780; ^1H NMR: 1.25 (3H, d, $J = 6.0$ Hz, CH_3), 1.87 (1H, br s, OH), 2.64–2.80 (2H, m, CH_2), 3.81 (1H, s, O–CH_3), 3.99–4.05 (1H, m, O–CH), 6.78–6.83 (3H, m, $Ar_{2,4,6}$–H), 7.21–7.27 (1H, m, Ar_5–H); ^{13}C NMR: 22.95, 46.00, 55.31, 68.95, 111.98, 115.27, 121.88, 129.69, 140.31, 159.91. Anal. Calcd for $C_{10}H_{14}O_2$: C, 72.26, H, 8.49. Found: C, 72.11, H, 8.54.

4.3.6. (*S*)-1-(3-Methoxyphenyl)propan-2-ol (*S*)-2d. Yield: 74%; ee: >99% (GC); $[\alpha]_D^{25} = +30.3$ (*c* 1.0, $CHCl_3$) {lit.:[13] $[\alpha]_D = +31.8$ (*c* 1.53, $CHCl_3$)}; The IR, ^1H and ^{13}C NMR spectra of (*S*)-**2d** were indistinguishable from that of *rac*-**2d**.

4.3.7. Racemic 1-(4-methoxyphenyl)propan-2-ol *rac*-2e. Yield: 88%; IR: 3360, 2968, 1616, 1512, 1248, 1148, 1036, 944, 808; ^1H NMR: 1.24 (3H, d, $J = 6.3$ Hz, CH_3), 2.63–2.78 (2H, m, CH_2), 2.96 (1H, br s, OH), 3.81 (1H, s, O–CH_3), 3.96–4.05 (1H, m, CH), 6.87 (2H, d, $J = 8.7$ Hz, $Ar_{2,6}$–H), 7.14 (2H, d, $J = 8.7$ Hz, $Ar_{3,5}$–H); ^{13}C NMR: 22.73, 44.92, 55.92, 69.22, 114.13, 116.21, 130.50, 158.41. Anal. Calcd for $C_{10}H_{14}O_2$: C, 72.26, H, 8.49. Found: C, 72.15, H, 8.38.

4.3.8. (*S*)-1-(4-Methoxyphenyl)propan-2-ol (*S*)-2e. Yield: 84%; ee: >99% (GC); $[\alpha]_D^{25} = +34.9$ (*c* 1.0, $CHCl_3$) {lit.:[14] $[\alpha]_D = +27.0$ (*c* 4.4, $CHCl_3$)}; ^1H NMR spectrum agreed with the reported data.[15] The IR, ^1H and ^{13}C NMR spectra of (*S*)-**2e** were undistinguishable from that of *rac*-**2e**.

4.3.9. Racemic 1-(2,4-dimethoxyphenyl)propan-2-ol *rac*-2g. Yield: 41%; IR: 3387, 2968, 1580, 1475, 1268, 1166, 1043, 944, 793; ^1H NMR: 1.21 (2H, d, $J = 6.3$ Hz, CH_3), 1.81 (1H, br s, OH), 2.64–2.8 (2H, m, CH_2), 3.8 (3H, s, O–CH_3), 3.81 (3H, s, O–CH_3), 6.45 (2H, dd, $J_1 = 7.8$ Hz, $J_2 = 2.2$ Hz, Ar_5–H), 6.45 (1H, br s, Ar_3–H), 7.05 (1H, d, $J = 7.8$ Hz, Ar_6–H); ^{13}C NMR: 23.10, 39.97, 55.53, 55.59, 68.27, 98.92, 104.35, 119.52, 131.80 158.69, 159.95. Anal. Calcd for $C_{11}H_{16}O_3$: C, 67.32, H, 8.22. Found: C, 67.35, H, 8.24.

4.3.10. (*S*)-1-(2,4-Dimethoxyphenyl)propan-2-ol(*S*)-2g. Yield: 38%; ee: >99% (GC); $[\alpha]_D^{25} = +19.7$ (*c* 1.0, $CHCl_3$); ^1H NMR spectrum agreed with the reported data.[16] The IR, ^1H and ^{13}C NMR spectra of (*S*)-**2g** were indistinguishable from that of *rac*-**2g**.

4.3.11. Racemic 1-(4-chlorophenyl)propan-2-ol *rac*-2h. Yield: 70%; IR: 3368, 2968, 1492, 1408, 1088, 1016, 936, 800; ^1H NMR: 1.22 (2H, d, $J = 6.25$ Hz, CH_3), 1.94 (1H, s, OH), 2.63–2.77 (2H, m, CH_2), 3.95–4.02 (1H, m, CH), 7.15 (2H, d, $J = 8.4$ Hz, $Ar_{2,6}$–H), 7.28 (2H, d, $J = 8.4$ Hz, $Ar_{3,5}$–H); ^{13}C NMR: 22.97, 45.09, 68.85, 128.72, 130.89, 132.39, 137.20. Anal. Calcd for $C_9H_{11}ClO$: C, 63.35, H, 6.50, Cl, 20.78. Found: C, 63.18, H, 6.48, Cl, 20.91.

4.3.12. (*S*)-1-(4-Chlorophenyl)propan-2-ol (*S*)-2h. Yield: 70%; ee: >99% (GC); $[\alpha]_D^{25} = +31.0$ (*c* 1.0, $CHCl_3$); The IR, ^1H and ^{13}C NMR spectra of (*S*)-**2h** were undistinguishable from that of *rac*-**2h**.

4.4. Synthesis of the (*S*)-1-phenylbutan-2-ol (*S*)-4a by ring opening of (*S*)-propylene oxide 7 with benzylmagnesium bromide

To a stirred solution of (6 mmol, 146 mg) magnesium, and one drop of 1,2-dibromoethane in diethyl ether (5 ml), benzyl bromide (6 mmol, 1.03 g) in 5 ml diethyl ether was added dropwise, and the reaction mixture stirred under reflux until the magnesium dissolved. Then (*S*)-propylene oxide (*S*)-**7** (3 mmol, 0.17 g, 0.21 ml) was added under −30 °C and the mixture stirred at this temperature for 20 min. Then, the temperature was raised up to room temperature (about 30 min), and then stirred for 1 h. The reaction mixture was quenched by the addition of NH_4Cl (8 ml) and diethyl ether (30 ml). The mixture was washed with water (8 ml), and brine (8 ml). The organic solution was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel; hexane–acetone 10:1) to yield the secondary alcohol (*S*)-**4a**.

The corresponding racemic alcohol *rac*-**4a** was synthesized from benzyl bromide, magnesium and racemic propylene oxide *rac*-**7** (3 mmol) in a similar reaction.

4.4.1. Racemic 1-phenylbutan-2-ol *rac*-4b. Yield: 57%; IR: 3344, 2968, 1496, 1456, 1076, 1032, 760, 680; ^1H NMR: 1.18 (3H, d, $J = 6.9$ Hz, CH_3), 1.55–1.99 (2H, m, CH_2), 2.23 (1H, br s, OH), 2.55–2.87 (2H, m, CH_2), 3.61–3.92 (1H, m, O–CH), 7.25–7.39 (5H, m, Ar–H); ^{13}C NMR: 10.83, 29.19, 43.2, 74.07, 126.63, 128.67, 129.55, 138.62. Anal. Calcd for $C_{10}H_{14}O$: C, 79.96, H, 9.39. Found: C, 79.83, H, 9.44.

4.4.2. (*S*)-1-Phenylbutan-2-ol (*S*)-4b. Yield: 57%; ee: >98% (GC); $[\alpha]_D^{25} = +12.3$ (*c* 1.0, $CHCl_3$) {lit.:[17] $[\alpha]_D = +17.2$ (*c* 1.0, $CHCl_3$)}; ^1H NMR spectrum agreed with the reported data ^1H data.[17] The IR, ^1H and ^{13}C NMR spectra of (*S*)-**4b** were indistinguishable from that of *rac*-**4b**.

4.5. Chiral HPLC and GC analysis and absolute configuration of the secondary alcohols 2b–e,g,h and 4a

The enantiomeric compositions of the secondary alcohols from bioreductions (**2a**–**h** and **4a**,**b** and **6**;

see Section 4.2) were determined by HPLC. The enantiomeric purity of the synthetic (*S*)-alcohols (*S*)-**2b–e,g,h** and (*S*)-**4a** were determined by GC. The absolute configuration of the bioreduction products **2b–e,g,h** and **4a** were determined by comparison with the synthetic (*S*)-alcohols (*S*)-**2b–e,g,h** and (*S*)-**4a** by HPLC.

4.5.1. 1-Phenylpropan-2-ol, 2b. GC: t_R (BetaDex 225; 120 °C)/min: 6.88 (*S*)-**2b** and 7.02 (*R*)-**2b** (baseline separation); HPLC: HPLC: t_R (mobile phase: 2% 2-propanol in hexane; $\lambda = 254$ nm)/min: 8.47 (*S*)-**2c** and 9.25 (*R*)-**2c** (baseline separation).

4.5.2. 1-(2-Methoxyphenyl)propan-2-ol, 2c. GC: t_R (Lipodex E; 100–200 °C, 10 °C/min)/min: 3.79 (*S*)-**2c** and 3.82 (*R*)-**2c** (baseline separation); HPLC: t_R (mobile phase: 5% 2-propanol in hexane; $\lambda = 277$ nm)/min: 2.10 (*S*)-**2c** and 3.27 (*R*)-**2c** (baseline separation).

4.5.3. 1-(3-Methoxyphenyl)propan-2-ol, 2d. GC: t_R (Lipodex E; 100–200 °C, 10 °C/min)/min: 5.77 (*S*)-**2d** and 6.00 (*R*)-**2d** (baseline separation); HPLC t_R (mobile phase: 5% 2-propanol in hexane; $\lambda = 277$ nm)/min: 5.37 (*S*)-**2d** and 5.96 (*R*)-**2d** (baseline separation).

4.5.4. 1-(4-Methoxyphenyl)propan-2-ol, 2e. GC: t_R (BetaDex 225; 150 °C)/min: 8.43 (*S*)-**2e** and 8.55 (*R*)-**2e** (baseline separation); HPLC t_R (mobile phase: 1% 2-propanol in hexane; $\lambda = 286$ nm)/min: 6.53(*S*)-**2e** and 6.90 (*R*)-**2e** (near-baseline separation).

4.5.5. 1-(2,4-Dimethoxyphenyl)propan-2-ol, 2g. GC: t_R (separation as acetate, prepared from **2g** by excess Ac_2O and Et_3N and heating; Lipodex E; 100–200 °C, 10 °C/min)/min: 7.85 (*S*)-**2g**-Ac and 8.14 (*R*)-**2g**-Ac (baseline separation); HPLC t_R (mobile phase: 10% 2-propanol in hexane; $\lambda = 286$ nm)/min: 2.76 (*S*)-**2g** and 3.16 (*R*)-**2g** (baseline separation).

4.5.6. 1-(4-Chlorophenyl)propan-2-ol, 2h. GC: t_R (separation as acetate, prepared from **2h** by excess Ac_2O and Et_3N and heating; Lipodex E; 100–150 °C, 2 °C/min)/min: 13.33 (*S*)-**2h**-Ac and 13.50 (*R*)-**2h**-Ac (baseline separation); HPLC: t_R (mobile phase: 1% 2-propanol in hexane; $\lambda = 286$ nm)/min: 5.82 (*S*)-**2h** and 8.86 (*R*)-**2h** (baseline separation).

4.5.7. 1-Phenylbutan-2-ol, 4a. GC: t_R (Lipodex E; 100–200 °C, 10 °C/min)/min: 2.85 (*S*)-**4a** and 2.93 (*R*)-**4a** (baseline separation); HPLC: t_R (mobile phase: 2% 2-propanol; $\lambda = 260$ nm)/min: 5.82 (*S*)-**2b** and 8.86 (*R*)-**2b** (baseline separation).

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